Paralytic Shellfish Poisoning (PSP) toxins in Miramichi Bay (New Brunswick, southern Gulf of St. Lawrence): Seeking the source

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PARALYTIC SHELLFISH POISONING (PSP) TOXINS IN MIRAMICHI BAY (NEW BRUNSWICK, SOUTHERN GULF OF ST. LAWRENCE): SEEKING THE SOURCE

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ABSTRACT

Léger, C.E. and S.S. Bates. 2012. Paralytic shellfish poisoning (PSP) toxins in Miramichi Bay (New Brunswick, southern Gulf of St. Lawrence): Seeking the source. Can. Manuscr. Rep. Fish. Aquat. Sci. 3002: v + 26 p.

A study was carried out to investigate the origins of irregular occurrences of Paralytic Shellfish Poisoning (PSP) toxins in molluscs in the Miramichi Bay (New Brunswick, Canada) area, where they were first recorded in 1988. PSP toxins are produced by the dinoflagellate *Alexandrium tamarense*, and may become concentrated in the flesh of filter-feeding molluscs. When toxin concentrations in molluscs exceed the regulatory limit, mollusc harvesting areas are closed to safeguard human health. Such closures, from 2001 to 2008, are documented in this report. Data from regulatory analyses were used to infer patterns of toxic bloom dynamics and timing. *Alexandrium tamarense* cells also produce cysts that settle to the bottom, where they lay dormant until conditions again become favourable for growth and a bloom is initiated. To evaluate *A. tamarense* cyst presence in sediments, sediment was sampled in Miramichi Bay and points north, during 2007 and 2009. However, in this study only a limited number of *A. tamarense* cysts were found in sediments from the 17 sites sampled. Phytoplankton was sampled near mollusc sampling sites, but no relation was found between numbers of *A. tamarense* cells in the water column and occurrences of toxins in molluscs, with one notable exception. Although the origins of the toxic blooms remain elusive, indirect evidence suggests that offshore *A. tamarense* blooms from the southern Gulf of St. Lawrence are swept into Miramichi Bay by tidal or other currents. A direct link between toxic blooms in Miramichi Bay and those in the St. Lawrence Estuary is not supported by the available evidence. We conclude that hydrodynamics complicates the relationships among cysts in bottom sediments, vegetative cells in the water and toxic bivalves. Hence, cyst mapping is unlikely to be of great use in Miramichi Bay and surrounding embayments.

RÉSUMÉ

Léger, C.E. and S.S. Bates. 2012. Paralytic shellfish poisoning (PSP) toxins in Miramichi Bay (New Brunswick, southern Gulf of St. Lawrence): Seeking the source. Can. Manuscr. Rep. Fish. Aquat. Sci. 3002: v + 26 p.

Une étude a été menée sur les origines des toxines du genre IPM (Intoxication Paralytique par les Mollusques), présentes de façon irrégulière dans les mollusques de la baie Miramichi (Nouveau-Brunswick, Canada), depuis leur première détection en 1988. Les toxines de l'IPM sont produites par le dinoflagellé *Alexandrium tamarense*, et peuvent se concentrer dans la chair de mollusques filtreurs, ce qui pose un danger à la santé humaine. Ainsi, lorsque les concentrations de toxines dans les mollusques atteignent la limite réglementaire les zones affectées sont fermées à la récolte de mollusques. De telles fermetures furent imposées dans la région de la baie Miramichi entre 2001 et 2008, et sont documentées dans le présent rapport. Les données d'analyses réglementaires des mollusques ont servi pour élucider la dynamique et la périodicité des blooms toxiques de *A. tamarense*. Des sédiments de la baie Miramichi et de quelques localités adjacentes furent prélevés en 2007 et 2009, pour évaluer la présence de kystes de *A. tamarense*, puisque cette espèce peut produire des kystes qui se déposent au fond et peuvent éclore et initier des blooms toxiques lorsque les conditions ambiantes deviennent favorable à leur multiplication. Cependant, dans cette étude peu de kystes ont été trouvées dans les sédiments prélevés à 17 sites. Le phytoplancton a été échantillonné près des sites d'échantillonnage de mollusques, mais aucun rapport n'a pu être établi entre la population de *A. tamarense* et la présence de toxines dans les mollusques, sauf pour une exception notable. Quoique les origines des toxines demeurent incertaines, les données suggèrent qu'il s'agirait de blooms de *A. tamarense* se formant au large des côtes, qui seraient amenés dans la baie Miramichi par les courants de marée ou autres courants. Les données disponibles n'indiquent pas de lien direct entre les blooms périodiques de *A. tamarense* dans la baie Miramichi et ceux de l'estuaire du St. Laurent. Nous concluons que les facteurs hydrodynamiques compliquent les rapports entre les kystes dans les sédiments, les cellules végétatives dans la colonne d'eau, et la présence de toxines dans les bivalves. Il semble donc improbable que la cartographie des kystes puisse s'avérer utile dans la baie Miramichi et les eaux environnantes.

1. Introduction

Although long known to be endemic to the Bay of Fundy / Gulf of Maine system, as well as to the St. Lawrence Estuary and the adjacent Gaspé coast, Paralytic Shellfish Poisoning (PSP) was not considered a threat to human health nor to the molluscan shellfish industry of Miramichi Bay prior to 1988 (Prakash et al. 1971, Bates 2005). Before that year there had been no documented occurrences of PSP in this area, and no records of blooms of *Alexandrium tamarense* (Fig. 1), the dinoflagellate that produces PSP toxins. Because of this, regulatory monitoring of the molluscan fishery for PSP toxins had been carried out at a minimal level. This was true also of the outlying areas of Northumberland Strait and of the southern Gulf of St. Lawrence (Fig. 2). However, in 1988 PSP toxins were discovered in several molluscan shellfish species in Miramichi Bay (Worms et al. 1993), and then again in the 2000s.

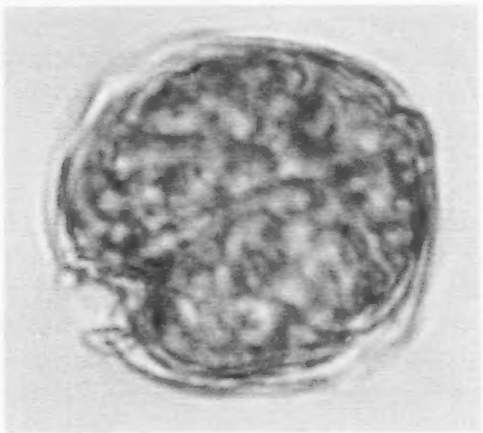

Figure 1. *Alexandrium tamarense* motile cell. Baie-Sainte-Anne, New Brunswick, 10 June, 2008. 400x magnification. The cell diameter is ~50 µm.

This report provides a synopsis of closures of shellfish harvesting due to PSP toxins in the southern Gulf of St. Lawrence and explores possible sources of the *A. tamarense* blooms in Miramichi Bay (Fig. 3), an important shellfish harvesting region along the eastern coast of New Brunswick.

The PSP toxins include saxitoxin (STX) and approximately two dozen naturally occurring derivatives of differing potencies (Lucas et al. 2003). These toxins specifically bind to sodium channels in vertebrate nervous systems, which blocks signal transmission to nerve and muscle cells, causing paralysis (hence the name Paralytic Shellfish Poisoning). In vertebrates, typical symptoms of poisoning include tingling and numbness of the lips and fingers, followed by muscle paralysis. In extreme cases, this can lead to death by asphyxiation. Toxin-producing *Alexandrium* cells can be filtered out of the seawater by molluscan (mainly bivalve) shellfish, which can then transfer the concentrated toxins to humans who consume them. The biology of the genus *Alexandrium* has recently been reviewed (Anderson et al. 2012). Note that the currently used name of the species found in the Gulf of St. Lawrence is *Alexandrium tamarense*. Previous nomenclature used in older literature includes: *Alexandrium excavatum*, *Protogonyaulax tamarensis*, *Gonyaulax tamarensis*, *Gonyaulax excavata*, *Gessnerium tamarensis*, and *Gonyaulax globosa*.

The concentration of PSP toxins in mollusc flesh is expressed as micrograms of saxitoxin equivalents (STXeq) per 100 g (wet weight) of mollusc meat, as per the AOAC method for PSP testing by mouse bioassay (AOAC 1990) and, more recently, by the post-column oxidation (PCOX) HPLC method (van de Riet et al. 2011).

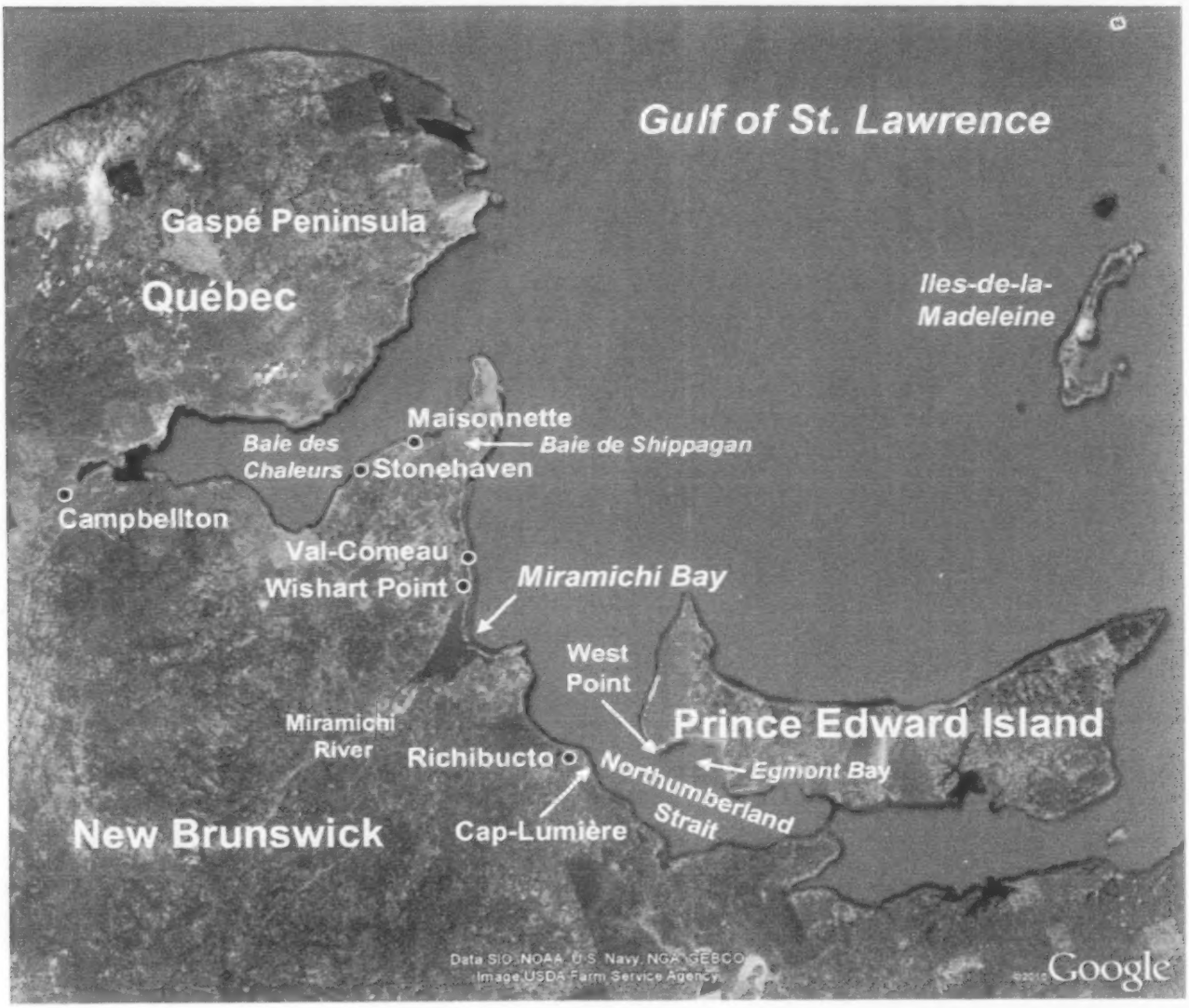

Figure 2. Southern Gulf of St. Lawrence and Northumberland Strait.

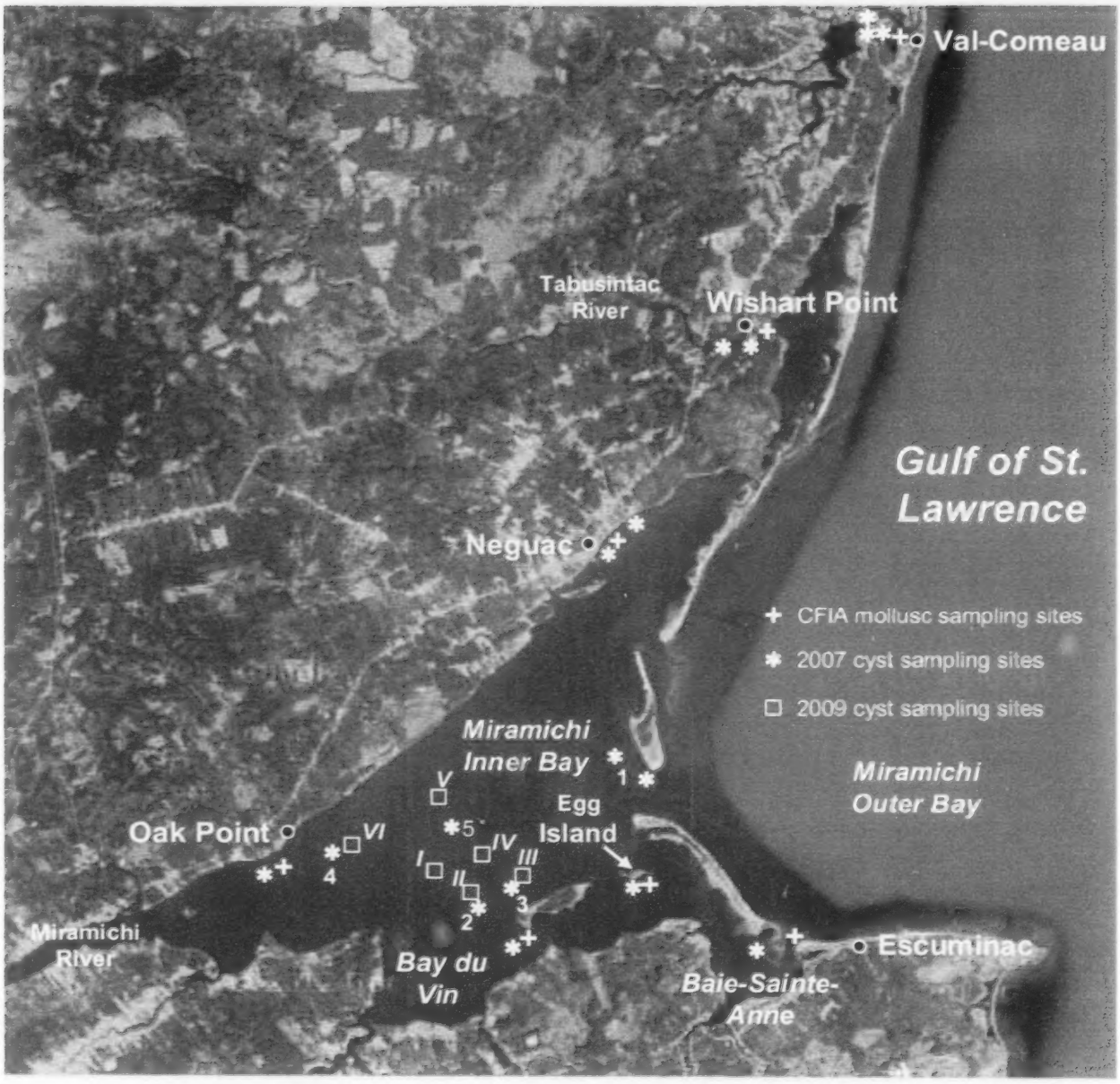

Figure 3. Sampling stations in Miramichi Bay, New Brunswick.

The limit of detection of the mouse bioassay is ~40 µg STXeq/100 g of wet tissue, and the Health Canada tolerance limit for human consumption of mollusc meat is established at 80 µg STXeq/100 g (or 80 mg STXeq/kg) (CSSP 2011). All of the numerical results cited in this report are from mouse bioassays carried out by the Inspection Branch of the Department of Fisheries and Oceans (DFO) and, after 1997, the Canadian Food Inspection Agency (CFIA).

The CFIA is responsible for testing mollusc meat for the presence of biotoxins, including PSP toxins, as part of the Canadian Shellfish Sanitation Program (CSSP). If biotoxin levels are approaching, or above, the regulatory limit, the CFIA makes a recommendation to DFO that an area be closed to molluscan shellfish harvesting. The Conservation and Protection Branch of DFO is then legally responsible for closing the affected area, patrolling it to insure that no harvesting occurs, and then reopening it after biotoxin levels fall to below the limit for the required period of time. Information about the control of marine biotoxins in Canada is found at http://www.inspection.gc.ca/english/fssa/fispoi/man/cssppccsm/chap11e.shtml.

Following the first documented discovery of PSP toxins in Miramichi Bay in 1988 (Worms et al. 1993), there was interest in developing tools to forecast future occurrences. This led to questions regarding the source of the PSP-causing dinoflagellate blooms affecting Miramichi Bay: are the blooms a local-scale phenomenon originating from cysts (a dormant stage; see Figs. 4–6); a regional offshore bloom advected inshore; or transported from further afield, i.e. from the St. Lawrence estuary, via the Gaspé current (e.g. Trites and Drinkwater 1991)? However, scientific interest in PSP in DFO's Gulf Region waned in the mid-1990s, because there were few additional molluscan shellfish harvesting closures due to PSP toxins.

Throughout the 1990s, most of the scientific attention concerning phycotoxins in the Gulf Region was focused on eastern and northern Prince Edward Island (PEI), where blooms of the chain-forming pennate diatom *Pseudo-nitzschia multiseries* (then designated as *Nitzschia pungens* f. *multiseries*) had caused outbreaks of the newly discovered neurotoxin, domoic acid, and the new syndrome it caused, Amnesic Shellfish Poisoning (ASP) (reviewed by Bates et al. 1998, Bates 2004, Bates and Trainer 2006, Lelong et al. 2012, Trainer et al. 2008, 2012). Human ASP intoxications and several deaths had occurred in the late autumn of 1987, following consumption of toxic mussels. This outbreak led to the subsequent wider surveillance for other phycotoxins in the area (Worms et al. 1993).

Another type of shellfish poisoning, Diarrhetic Shellfish Poisoning (DSP), also became a concern just prior to this period, because some dinoflagellate species known to cause it (*Dinophysis* spp., *Prorocentrum* spp.) are commonly found in waters of the southern Gulf of St. Lawrence (Cembella 1989, Luu et al. 1993, Levasseur et al. 2003). It was known that DSP toxins are a major cause of shellfish closures in Europe, Japan and South America (reviewed by Reguera et al. 2012).

In the mid-1990s, oceanographic research expanded to the Acadian Peninsula of the northeastern coast of New Brunswick, which encompasses the outer Miramichi Bay (Fig. 3), where biological, chemical and physical oceanographic sampling was carried out between 1991 and 1996 (Waite et al. 1997a, b, c, d, 1998a, b). No blooms of toxin-producing species were recorded in those studies, and plankton species are not listed in the published reports.

In the early 2000s, interest in the source of the Miramichi Bay PSP toxins was rekindled, with the reappearance of toxic *Alexandrium* blooms. These blooms forced temporary closures of the molluscan fisheries in the Miramichi Bay area, and further south along the northwestern Northumberland Strait coasts of both New Brunswick and PEI (Fig. 2, Table 1, Appendix Table A1).

Table 1. Closures of molluscan shellfish harvesting along the eastern New Brunswick (NB) and Northumberland Strait coasts, southern Gulf of St. Lawrence, caused by levels of PSP toxins above the regulatory limit (80 µg STXeq/100 g). Where values are shown as <80 µg STXeq/100 g, the closure was precautionary. Levels are shown for blue mussels (*Mytilus edulis*), unless otherwise noted. Some of the closures and re-openings are actually more complicated that those shown in this table, because of partial re-openings and broadening of closures in some locations. When available, links are given to the Prohibition Order (underlined). See Appendix Table A1 for the complete list of Prohibition Orders.

Location	Date Closed	Prohibition Order	Date Opened	Prohibition Order	Days Closed	Max PSP toxin conc. (µg STXeq/100 g)
Baie-Sainte-Anne, NB	27-Jun-00	GTN-2000-01	3-Aug-00	GTN-2000-02	37	270
West Point to Seal Point, PEI	17-Jun-03	GTN-2003-001	17-Jul-03	GTN-2003-004	30	145 (bar clams)
Egmont Bay, PEI	23-Jun-03	GTN-2003-002	1-Aug-03	GTN-2003-006	39	-
Miramichi Bay, NB	23-Jun-04	GTN-2004-001	22-Jul-04	GTN-2004-002	29	317
Miramichi Bay and section of Gloucester Co., NB	09-Jun-06	GTN-2006-001	06-Jul-06	GTN-2006-006	27	223
Broadened to Shippagan to Escuminac, NB	14-Jun-06	GTN-2006-002	06-Jul-06	GTN-2006-005	21	4,368
Broadened to Campbellton Bridge to Escuminac, NB	15-Jun-06	GTN-2006-003	06-Jul-06	GTN-2006-006	21	940
West Point to Seal Point, PEI	22-Jun-06	GTN-2006-004	10-May-07	GTN-2007-001	322	230
Brantville to Shippagan Gully, NB	12-Jul-06	GTN-2006-007	03-Aug-06	GTN-2006-009	22	-
Escuminac Point to Cap-Lumière, NB	01-Jun-07	GTN-2007-002	07-Jun-07	GTN-2007-005	6	91
Escuminac Point to Pokemouche Beach, NB	07-Jun-07	GTN-2007-004	06-Jul-07	GTN-2007-010	29	-
West Point to Seal Point, PEI	08-Jun-07	GTN-2007-006	06-Jul-07	GTN-2007-009	28	-
Escuminac Point to Cap-Lumière, NB	22-May-08	GTN-2008-001	11-Jun-08	GTN-2008-006	20	99
Portions of Miramichi Bay, Pokemouche Beach to Chamberlain Point, NB	30-May-08	GTN-2008-002	20-Jun-08	GTN-2008-008	21	93

2. Synopsis of sampling and analysis of molluscs in Miramichi Bay

The following is a brief synopsis of activities undertaken by Gulf Region's Oceans and Science Branch, aimed at identifying the source of PSP toxins in Miramichi Bay. Activities included observations of historical and annual trends of the presence of PSP toxins, seasonal monitoring of phytoplankton, as well as a search for resting cysts (a life stage; see below; Figs. 4–6) of the toxin-producing *Alexandrium* in bottom sediments.

2.1. Early sampling and analysis of molluscs (1988–1992)

Following the major domoic acid contamination event (causing ASP), which occurred in the autumn-winter 1987–1988, and affected cultured mussels in eastern PEI, an extensive new molluscan monitoring program was undertaken throughout the Gulf Region by DFO Inspection Branch, now part of the CFIA since April 1997. Its goal was to detect and measure levels of phycotoxins (PSP, ASP, DSP) throughout the southern Gulf of St. Lawrence, as a safety measure to protect human consumers of molluscan shellfish (Worms et al. 1993, Bouchard-Steeves et al. 1993a, b, c, d).

In Miramichi Bay and estuary (designated as Inspection areas 44 and 54), commercial molluscs were sampled weekly at six stations throughout the open water season of 1988. The mollusc species found to be most contaminated by PSP toxins was the bar clam (*Spisula solidissima*), in which the PSP concentration rose to 1,011 µg STXeq/100 g in early July, then decreased to about 40 µg STXeq/100 g by late August. Among the other exploited species sampled and analyzed, only one soft-shell clam (*Mya arenaria*) sample contained a high level of PSP toxins (190 µg STXeq/100 g). Mussels (*Mytilus edulis*), bay quahaugs (*Mercenaria mercenaria*) and oysters (*Crassostrea virginica*) contained levels of 62, 56 and 43 µg STXeq/100 g, respectively, which were all below the Health Canada tolerance limit.

Sampling continued in 1989, and the highest PSP toxin levels recorded were in scallops (*Placopecten magellanicus*) and moon snails (*Lunatia heros*). One sample of scallop (presumably viscera) contained 507 µg STXeq/100 g. Moon snails averaged 129 µg STXeq/100 g, with no marked variation over the sampling season, from June to November. It should be noted that moon snails had not been sampled in 1988, and were sampled in 1989 only after interest was expressed in commercial fishing of this species. PSP toxin levels in bar clams never exceeded 44 µg STXeq/100 g in 1989, and were negligible in other species sampled.

In 1990 and 1991, the only species found to be contaminated were moon snails, with levels surpassing 300 µg STXeq/100 g in 1990, and > 100 µg STXeq/100 g in 1991. Moon snails are principal predators of bar clams, which most likely accounts for the high accumulation of PSP toxins in their flesh. Moreover, these carnivorous gastropods depurate the toxin slowly from their flesh, in contrast to the more rapid depuration rates in most filter-feeding bivalves (reviewed by Deeds et al. 2008).

In 1992, extensive sampling of soft-shell clams, oysters and mussels showed no trace of PSP toxins in Miramichi Bay. However, neither moon snails nor bar clams were sampled in Miramichi Bay that year. It should also be noted that low levels of PSP toxins were detected that

year in moon snails further north, in the region of Maisonnette and Shippagan (Fig. 2), but not in bar clams (Bouchard-Steeves et al. 1993d).

The above information suggests that the mollusc species most exposed to the toxin-producing phytoplankton in Miramichi Bay were species inhabiting the more seaward areas of the bay and estuary: scallops growing in deeper offshore waters, and bar clams and moon snails generally found on subtidal or intertidal sand bars in open or relatively exposed areas. Fewer occurrences, and much lower PSP toxin levels, were found in the inshore species (soft-shell clams, oysters, mussels), suggesting a lower exposure to the toxin source in those areas. These observations suggest an offshore origin for the toxin-producing organism.

2.2. Recent sampling and analysis of molluscs (2002–2011)

There was no marked occurrence of PSP toxins in the Miramichi region after 1991, and as previously noted most of the attention regarding phycotoxins through the mid-1990s was directed at the recurring problem of ASP (domoic acid) in PEI, notably in 1994. There was also a widespread closure of southern Gulf waters, due to domoic acid (this time produced by *Pseudo-nitzschia seriata*), in April and early May, 2002, which included Miramichi Bay (Bates et al. 2002).

PSP toxins returned to Miramichi Bay and adjacent areas in the early 2000s, with a closure imposed in the Baie-Sainte-Anne area from 27 June to August 3, 2000 (Table 1). Results for the toxin analysis of molluscs indicate a maximum value of 270 µg STXeq/100 g during the closure, and that values had fallen to "acceptable values" by mid-July.

In 2001, a brief closure was imposed on June 8, for the waters of Miramichi Bay. However the area was reopened one day later, which indicates the closure was precautionary, and the levels of PSP toxins remained below the 80 µg STXeq/100 g tolerance limit.

In the summer of 2004, the waters of Miramichi Bay were closed after PSP toxins were detected in mussels placed in bottom cages by the CFIA for the purpose of sampling. The results that follow are all from CFIA mouse bioassay analyses and, unless otherwise indicated, the results are from analyses of mussel (*Mytilus edulis*) tissue.

Mussels sampled on 21 June 2004, at the Baie-Sainte-Anne sampling station (Fig. 3), had a PSP level of 317 µg STXeq/100 g. The levels declined rapidly to below the detection limit by early July. Samples from two other sites in more sheltered areas were at or below detection levels (< 40 µg STXeq/100 g).

A major closure of Miramichi Bay and the neighbouring coast occurred in June, 2006. PSP toxins were first detected at Val-Comeau on June 8, where the toxin level was 223 µg STXeq/100 g, and upriver at Oak Point, where they attained 190 µg STXeq/100 g. Both sites are on the north shore of the bay, and Oak Point is in the inner bay adjacent to the main river channel. No toxins were detected at that time elsewhere in the Miramichi area (Wishart Point, Egg Island, Bay du Vin, Baie-Sainte-Anne) or further down the Northumberland Strait coast.

The following week (June 14), the toxins reached a record high (4,368 µg STXeq/100 g) at Val-Comeau, but disappeared from Oak Point. However, toxins were now also present across the bay, at Baie-Sainte-Anne, peaking at 940 µg STXeq/100 g, whereas the other Miramichi sampling sites were still devoid of any toxins. Depuration of toxins from mussels at Val-Comeau occurred slowly over the following five to six weeks, until late July. In Baie-Sainte-Anne, where toxin levels were lower, it took three weeks. Elsewhere, toxins were also recorded concurrently at Stonehaven, in the Baie des Chaleurs, and in the Baie de Shippagan (in soft-shell clams), but at lower concentrations.

In 2007, PSP toxins returned to the Miramichi area, again in early June (Table 1), and were again detected first at Val-Comeau and Baie-Sainte-Anne. Toxin levels were much lower than in 2006, just surpassing the regulatory limit at Val-Comeau (91 µg STXeq/100 g), and just attaining the detection limit at Baie-Sainte-Anne (41 µg STXeq/100 g). Again, the other Miramichi Bay sampling sites were found to be devoid of toxins.

In 2008, the situation was repeated, although this time starting in mid-May (Table 1). PSP toxins were found at Val-Comeau (67 µg STXeq/100 g) and one week later at Baie-Sainte-Anne (58 µg STXeq/100 g). However, whereas the toxins had disappeared from Val-Comeau two weeks later (early June), they increased to above the regulatory limit at Baie-Sainte-Anne (93 µg STXeq/100 g), before declining to below the detection limit by mid-June. Again, as in the previous two years, no toxins were detected at the other Miramichi Bay sampling sites.

In 2008, PSP toxins were also detected further down the Northumberland Strait, at Cap-Saint-Louis and the Richibucto area (Fig. 2). Levels surpassed the regulatory limit only briefly (99 µg STXeq/100 g).

In 2009, no toxins were detected throughout the spring to autumn sampling season. Likewise, no toxins were detected in 2010, 2011, or 2012 (as of 22-Aug-12).

In assessing the foregoing observations pertaining to Miramichi Bay and estuary, the salient feature is the repeated appearance of PSP toxins first at Val-Comeau and Baie-Sainte-Anne (with the exception of the early Oak Point detection in 2006), with higher levels at these two stations. These sampling stations are the most seaward of the Miramichi Bay sites, and therefore the most exposed to the open waters of the Gulf. The stations are also located within channels with strong tidal currents carrying offshore water on incoming tides. The other stations, in contrast, are situated in more protected areas, landward. In support of the above, this again suggests an offshore source of the PSP-producing organism, as opposed to a purely local bloom originating within Miramichi Bay.

2.3. Phytoplankton observations and its relationship with toxins in mussels

In 1989, concurrent with the new molluscan monitoring program, a phytoplankton monitoring program was initiated by DFO Gulf Region in shellfish-producing areas to monitor populations of ASP-producing diatoms as well as PSP- and DSP-producing dinoflagellates. This phytoplankton watch was intended as an early warning tool to guide and complement the newly

increased regulatory sampling of shellfish for direct toxin analysis of the meat. It also provided information for aquaculturalists, who could then make management decisions. For example, they would be forewarned about possible harvesting closures if toxic phytoplankton concentrations began to increase. Conversely, they could anticipate a re-opening of the harvest area if the phytoplankton counts began to decrease. Indeed, toxic phytoplankton counts are currently used as a guide for closure decisions in other countries of the world (Anderson et al. 2001). However, this phytoplankton monitoring program was discontinued in the Gulf Region in the early 1990s, because it was deemed that regulatory closures in Canada are based solely on levels of phycotoxins in the shellfish flesh consumed by humans, as mandated by the CSSP (2011). As well, phytoplankton monitoring was discovered to be less informative as an early warning for harvesting closures caused by PSP-toxins in the Gulf Region (see below).

It should be noted that *A. tamarense* is generally found at only low concentrations in seawater samples from southern Gulf of St. Lawrence waters. An exception was the only significant bloom of this organism documented in Miramichi Bay, at Val-Comeau, on June 14, 2006, when numbers of *A. tamarense* cells were estimated at between 1,350 and 1,500 cells/litre. In this instance, the seawater was sampled simultaneously with the mussels containing the highest level of PSP toxins recorded (4,368 µg STXeq/100 g), as noted above.

From 2002 to 2009, regular phytoplankton sampling (by CFIA) and observations (by DFO Gulf Region) were again carried out at several areas of eastern New Brunswick, including Miramichi Bay. Initially, this was to find and isolate toxin-producing species (especially the domoic-acid-producing *Pseudo-nitzschia multiseries* and *P. seriata*) for use in laboratory research. This evolved into a routine phytoplankton monitoring activity, to observe trends in phytoplankton species composition and rough abundance. Although *A. tamarense* cells were observed frequently, no link was established between their presence and the appearance of PSP toxins in the flesh of sampled molluscs. One notable exception was the June 14, 2006 sampling at Val-Comeau, noted above.

Alexandrium tamarense is commonly found in seawater samples throughout the southern Gulf of St. Lawrence. It was generally thought to be a spring-summer species, but the highest recorded concentration in Gulf waters was found unexpectedly in a sample collected beneath the ice in the Brudenell River estuary, eastern PEI, in February 1990, where cell concentrations attained 10,000 cells/litre (Pauley et al. 1993). Thus, contamination of molluscan shellfish by PSP toxins may come at unexpected times of the year, although this has not yet been documented again.

Such high concentrations of *Alexandrium* cells, however, have rarely been encountered in southern Gulf of St. Lawrence waters, and the occurrences of PSP-causing blooms have been deduced only from the toxins in molluscs sampled for regulatory purposes. *Post-hoc* seawater sampling of contaminated areas has usually turned up only a few motile *A. tamarense* cells or none at all. Moreover, with the exception of the June 14, 2006 sample noted above, seawater samples obtained simultaneously with molluscs found to be toxic generally yielded few if any of the toxin-producing organisms. This discrepancy has been noted before (Cembella et al. 1988, Smith and Pauley 1990), and has raised questions about the value of seawater sampling of phytoplankton for predicting the occurrence of PSP toxin outbreaks. It is likely that such

considerations also led to the abolition of DFO's phytoplankton monitoring program of the early 1990s, mentioned above.

The undertaking of extensive and costly phytoplankton surveys is therefore not a promising direction as pertains to the scientific question of the origin of the PSP-producing blooms, at least in areas where such blooms are sporadic in nature. It should nevertheless be pointed out that phytoplankton monitoring has been useful in the case of giving an advance warning to the mussel aquaculture industry of impending domoic-acid-producing *Pseudo-nitzschia* blooms on PEI (Bouchard-Steeves et al. 1993a). Such a program, begun in 1990 after ASP first manifested itself in late 1987 with disastrous consequences to the shellfish industry, continues to be carried out by the provincial government (e.g. Smith and Ramsay 2011).

2.4. Dinoflagellate resting cysts

One tool that has been applied to understand the origin of *Alexandrium* blooms, and to forecast their occurrence, is the analysis of the distribution and concentration of *Alexandrium* cysts (Fig. 4) in sediments. Figure 5 illustrates the two types of cysts that are produced by dinoflagellates as part of their life cycle (Anderson 1998). One type, the hypnozygote, forms as a result of sexuality; gametes are produced and fuse to form swimming zygotes (planozygotes), which in turn encyst, becoming dormant, resting cysts (hypnozygotes). Another type, the temporary cyst, forms when conditions become adverse to further vegetative growth. Both types are resilient, dense cells that sink to the sediments, where they remain until conditions (usually temperature, light and oxygen, but there are also endogenous triggers) again become favourable for growth (Fig. 6). After germination (excystment), flagellated cells swim to the upper water column, where they continue to divide vegetatively. Thus, cysts can be a reservoir, or seed population, that can initiate dense blooms, hence their interest to scientists.

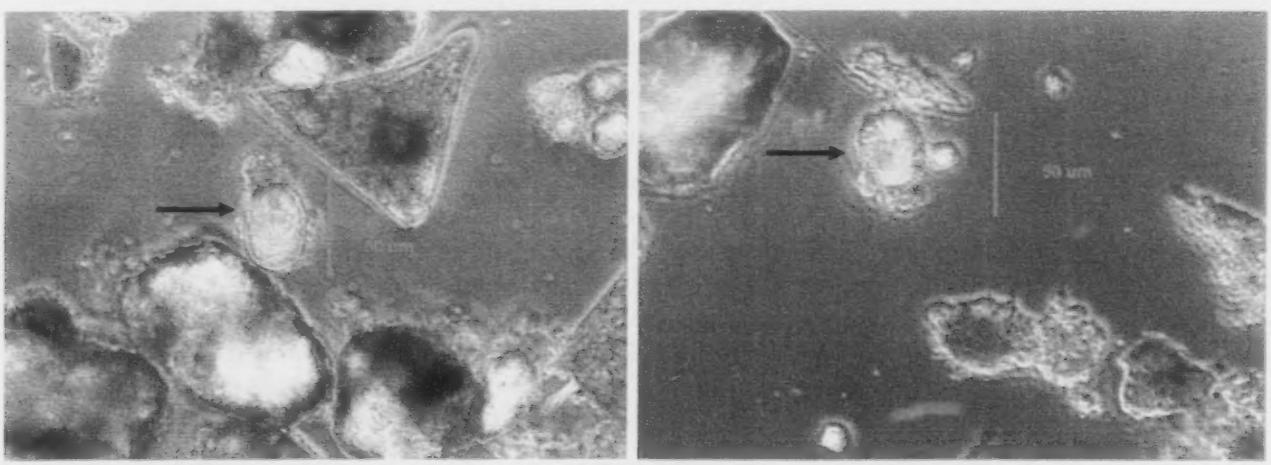

Figure 4. *Alexandrium tamarense* resting cysts (shown by an arrow in the left and right panels). Miramichi Bay, 2009. The scale bars are 50 µm.

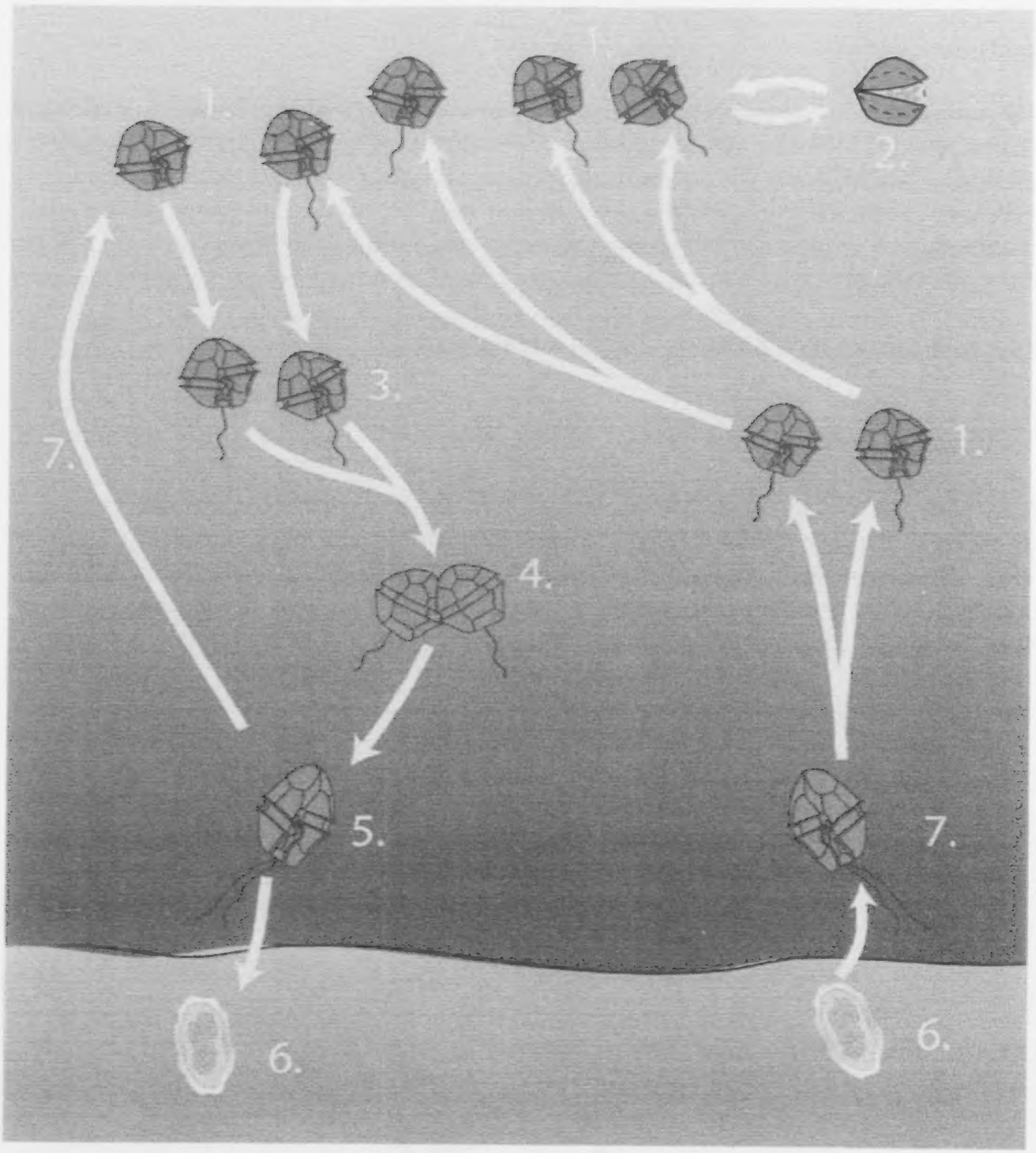

Figure 5. Schematic representation of the life cycle of heterothallic *Alexandrium* species. Species have a haplontic life cycle, i.e. the motile vegetative cells (1) are haploid. Under specific conditions, usually related to stress, some vegetative cells can transform into a non-motile pellicle cyst (2) that can rapidly switch back to the motile stage when conditions improve. The sexual phase starts with the formation of gametes (3), which conjugate (4) and form a diploid planozygote (5). Depending on environmental conditions, the planozygote can transform into a resting cyst (hypnozygotes) (6) or, for some species, it can undergo meiosis and produce a vegetative cell (1). Cysts can spend variable periods of time in the sediments and, upon germination, release a motile cell (7). Figure and text from Anderson et al. (2012).

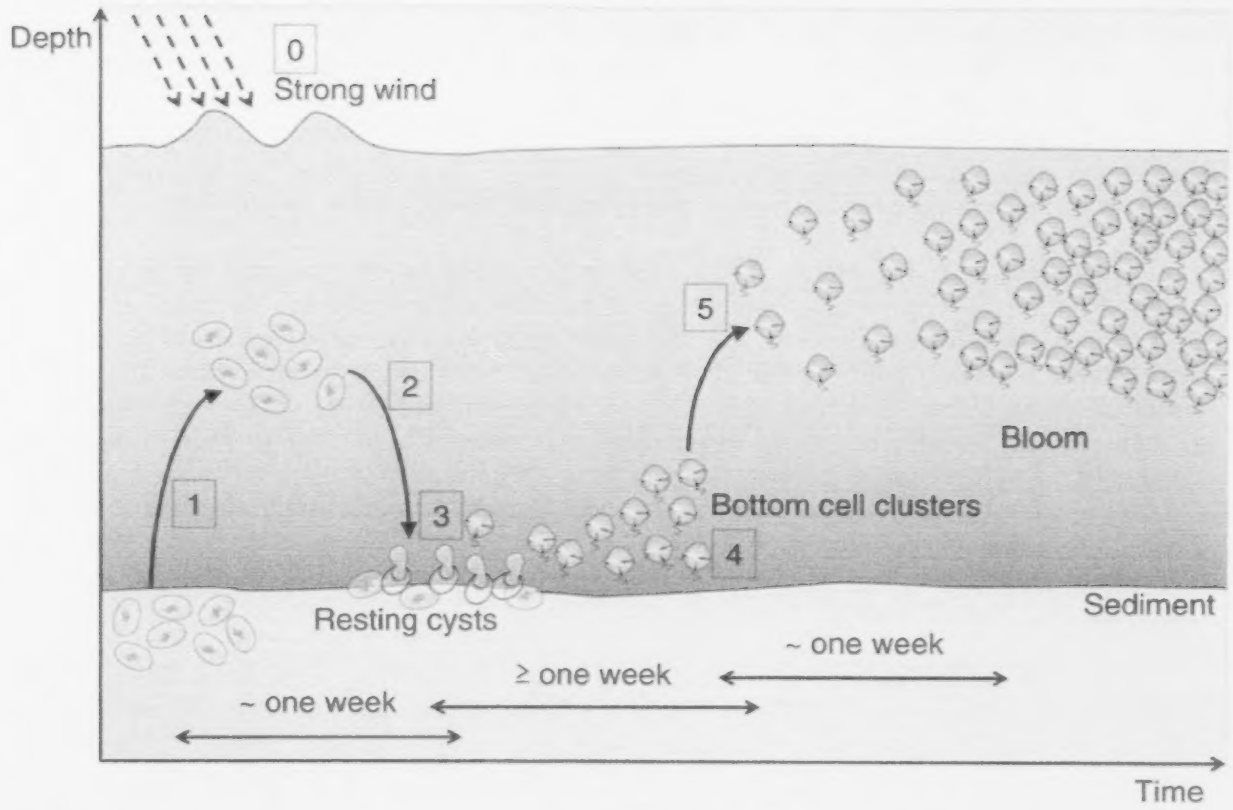

Figure 6. Schematic of bloom development from cyst banks in a shallow coastal environment. The scenario is based on a study of *A. tamarense* in Thau Lagoon, southern France (Genovesi et al. 2009), so that the timing and other details shown may be different for the southern Gulf of St. Lawrence. Strong winds (0) result in turbulent re-suspension of cysts (1), which allows cysts to be released from sedimentary anoxic conditions and exposed to light. Cysts eventually settle to the sediment after the wind stops (2). Synchronized germination of cysts occurs while seawater temperature rises (3). Cells proliferate slowly near the sediment surface (4), awaiting optimum growth conditions (5) As hydrological conditions become optimal for growth, upward migration and rapid cell proliferation lead to the formation of a bloom (5). Figure and text from Genovesi et al. (2009).

Mapping the distribution and concentration of these cysts may therefore be used to predict the location and severity of subsequent blooms. This approach has been previously applied, with varying degrees of success, at several locations: Bay of Fundy (White and Lewis 1982, Martin and Wildish 1994); Gulf of Maine (Anderson et al. 2005a, b, McGillicuddy et al. 2011) and surrounding marsh systems (Crespo et al. 2011); Puget Sound, Washington State (Cox et al. 2008); Thau Lagoon, southern France (Genovesi et al. 2009); Tarragona Harbor, Catalan coast of Spain (Bravo et al. 2008); as well as the northern shore of the lower St. Lawrence Estuary

(Cembella et al. 1988). At some of these locations, cysts have been found to be the source of successive annual blooms; once *Alexandrium* has bloomed, their cysts may become the seeds for the following year's bloom. Smith and Pauley (1990) suggested that surveys of *A. tamarense* resting cysts can thus be used as a means of predicting toxic blooms of this dinoflagellate in the southern Gulf of St. Lawrence. An even greater predictor of the intensity of toxic *Alexandrium* blooms, in addition to cyst density surveys, is the measurement of the excystment and encystment stages of the life cycle (Anglès et al. 2012). This, of course, is much more labour intensive and requires the deployment of emergence and sediment traps, respectively.

The following is an example of how a recent cyst study has provided information about the genetic structure of *A. tamarense* populations in the Gulf of St. Lawrence. Although it was not used to forecast impending blooms, the study does delineate areas where cysts are present and absent in the southern Gulf. In June 2005, in the course of a major research cruise, the surface sediments of the Estuary and Gulf of St. Lawrence were sampled to study the genetic variability of *A. tamarense* throughout the St. Lawrence system (Hamel et al. 2007). Using a box core sampler or a Van Veen grab, sediment samples were obtained at six stations along the Laurentian Channel from Anticosti Island to Cabot Strait, and a further 16 samples were obtained at stations through the Northumberland Strait from Saint Georges Bay (Nova Scotia) to the outer Miramichi Bay, and into the Baie-des-Chaleurs. (The St. Lawrence Estuary and seaward areas as far as Gaspé Bay had also been sampled in September 2004.)

The results of the above cruise and subsequent genetic analyses indicated a single genetic population of *A. tamarense* throughout the Estuary and Gulf, contrary to a previous hypothesis that proposed the existence of two distinct populations (Anderson et al. 1994). Of particular interest for the present document, however, is that of the 29 stations sampled in 2005 (and 13 stations sampled in 2004), the seven stations located between the western approach to Northumberland Strait to the mouth of the Baie-des-Chaleurs, including three stations in outer Miramichi Bay and one offshore from Val-Comeau, were devoid of any *A. tamarense* cysts, as was a station on the Magdalen Islands (sampled in 2004). All the other stations throughout the St. Lawrence system showed the presence of *A. tamarense* cysts in greater or lesser quantities, the greater quantities being found in the St. Lawrence Estuary, as might be expected.

In June of 2006, one year after the cyst distribution study of Hamel et al. (2007), the major *A. tamarense* bloom (described on page 9, above) occurred in Miramichi Bay, at Val-Comeau. This lead to the postulate that this bloom may have deposited cysts in the inner portions of the Bay, which might serve as a seedbed that could give rise to subsequent blooms and closures due to PSP toxins in Miramichi Bay.

Thus, in 2007, an exploratory series of sediment sampling was undertaken by the first author of the present study at various locations of inner and outer Miramichi Bay, to identify and evaluate the population of *A. tamarense* resting cysts. The sampling was carried out with the assistance of CFIA molluscan sampling personnel, as well as with DFO Conservation and Protection personnel. The sampling sites are indicated in Figure 3.

3. Methodology

In 2007, 16 sites in the Miramichi Inner and Outer Bay (Fig. 3) were sampled between late July and early October. Sediment samples were obtained from the seabed using a Ponar sediment grab, and surface sediments were subsampled directly from the Ponar grab, using a flat laboratory spatula. Two shallow-water sites were sampled directly from the bottom by hand, using a large spatula. In 2009, all the sampling was carried out on a single field trip, in the Inner Bay area, in September. A Van Veen grab was used in 2009. From each grab sample a 40 cm^2 area of sediment, to a depth of 2 cm, was transferred from the grab to screw-capped plastic containers, as were the shallow water samples taken by hand directly from the bottom. All samples were returned to the laboratory in a cooler with ice, and stored in a refrigerator at 4°C.

In the laboratory, subsamples of 1 cm^3 were pre-washed with filtered seawater on a 400-µm (0.4 mm) Nitex screen to remove larger material. They were then washed with filtered seawater on a 100-µm Nitex screen, and the finer fraction was washed on a 20-µm Nitex screen. The three fractions (> 100 µm; 20–100 µm; and < 20 µm) were examined in petri plates on an inverted microscope, with most of the effort concentrated on the 20–100 µm fraction. Given that the *A. tamarense* cysts have a dimension of 40 to 50 µm, all of the cysts found were in the latter fraction.

4. Results

Examples of *Alexandrium tamarense* cysts found are shown in Figure 4. A summary of results is given in Table 2. As expected, few or no cysts were found in sand or gravel bottoms. As well, none were found in eel grass flats, although these were not sampled extensively. However, cysts were found in fine silt sediments of the main channel and subtidal mudflats of the inner Miramichi Bay. The resting cysts of *A. tamarense* fall into the 40 to 50 µm size range (0.04–0.05 mm) and may be expected to accumulate in muddy sediments containing similar-sized grains.

Unfortunately, there were insufficient numbers of *A. tamarense* cysts to evaluate statistically the population density. Difficulties with equipment at the time of sampling further reduced the statistical value of the samples, so that any results can only be considered semi-quantitative.

In 2009, the second series of bottom sampling was carried out using a larger (Van Veen) sediment grab, in the inner Miramichi Bay areas where cysts had been found in 2007. Several samples contained some *Alexandrium* cysts, but again not enough to permit any statistical analysis. However, it was noted that the 2009 samples contained fewer cysts than the 2007 samples. There is no evidence, therefore, that resting cysts form seed populations that are the source of PSP-causing dinoflagellate blooms in Miramichi Bay.

It should be noted that the 2007 sampling occurred only one year after the major *A. tamarense* bloom of June 2006. Although not abundant, cysts were found in the sediments examined. However, the 2007 and 2008 blooms were of a minor nature, so were not expected to yield many cysts in the 2009 sediment samples, as turned out to be the case.

Table 2. Summary of observations of the presence (+ indicates a greater number of cysts were present) or absence (ND) of *Alexandrium* cysts at stations in Miramichi Bay and points north. Figures 2 and 3 show location of sites; stations identified by place names are in Fig. 3; Inner Bay stations are identified by numbers; roman numerals indicate 2009 stations.

Site 2007	Station Number	Date Sampled	Sediment type, water depth	*Alexandrium* cysts
Baie-Sainte-Anne, Hardwicke	n/a	23-Jul-07	Sand-mud, 2.5 m	ND
Bay du Vin	n/a	23-Jul-07	Sand-mud, 2 m	ND
Miramichi Bay, Egg Island	n/a	23-Jul-07	Sand, 1.8 m	ND
Miramichi Bay, Oak Point	n/a	20-Sep-07	Mud, 1.8 m	ND
Neguac	n/a	20-Sep-07	Sand, 1.8 m	ND
Val-Comeau, shore, south end of highway bridge	n/a	20-Sep-07	Sand-mud, 0.3 m	Present +
Val-Comeau, shore, north end of highway bridge	n/a	20-Sep-07	Sand, 1.3 m	ND
Tabusintac, channel	n/a	25-Sep-07	Mud, 3 m	ND
Tabusintac, Wishart Pt. (a)	n/a	25-Sep-07	Sand-mud, 5 m	ND
Tabusintac, Wishart Pt. (b)	n/a	25-Sep-07	Sand-mud, 3 m	ND
Val-Comeau, Ferguson Pt. (a)	n/a	1-Oct-07	Mud, 2.5 m	ND
Val-Comeau, Ferguson Pt. (b)	n/a	1-Oct-07	Mud, 2.6 m	ND
Miramichi Bay	1	5-Oct-07	Sand, 6 m	ND
Miramichi Bay	2	5-Oct-07	Mud, 6.6 m	Present +
Miramichi Bay	3	5-Oct-07	Mud, 6.3 m	ND
Miramichi Bay, subsurface	4	5-Oct-07	Mud, 6.3 m	ND
Miramichi Bay, surface	4	5-Oct-07	Mud, 6.3 m	Present +
Miramichi Bay	5	5-Oct-07	Mud, 5.5 m	ND
2009				
Miramichi Bay	I (a)	14-Oct-09	Mud, 4.6 m	ND
Miramichi Bay	I (b)	14-Oct-09	Mud, 4.6 m	ND
Miramichi Bay	I (c)	14-Oct-09	Mud, 4.6 m	ND
Miramichi Bay	I (d)	14-Oct-09	Mud, 4.6 m	ND
Miramichi Bay	II (a)	14-Oct-09	Mud, 5.2 m	Present
Miramichi Bay	II (b)	14-Oct-09	Mud, 5.2 m	ND
Miramichi Bay	II (c)	14-Oct-09	Mud, 5.2 m	ND
Miramichi Bay	II (d)	14-Oct-09	Mud, 5.2 m	ND
Miramichi Bay	III	14-Oct-09	Mud, 6.2 m	ND
Miramichi Bay	IV (a)	14-Oct-09	Mud, 5.5 m	Present
Miramichi Bay	IV (b)	14-Oct-09	Mud, 5.5 m	Present +
Miramichi Bay	V	14-Oct-09	Mud, 5.2 m	ND
Miramichi Bay	VI (a)	14-Oct-09	Mud, 5.4 m	ND
Miramichi Bay	VI (b)	14-Oct-09	Mud, 5.4 m	Present

5. Discussion and conclusions

This study produced no hard evidence that *Alexandrium tamarense* cysts from within Miramichi Bay were the origin of the PSP-causing dinoflagellate blooms in that bay. Our conclusion is based on the paucity of *Alexandrium* cysts from the sediment surface of Miramichi Bay in 2007 and 2009, even after a bloom had occurred there in 2006 that resulted in the closure of molluscan harvesting due to unacceptable levels of PSP toxins. This conclusion is also supported by the lack of *Alexandrium* cysts in an area offshore of Miramichi Bay, in a previous study (Hamel et al. 2007). Others have shown that, in enclosed waters with little exchange from the offshore, resident populations of *Alexandrium* may "re-seed" themselves with cysts from a previous bloom (e.g. Cox et al. 2008, Genovesi et al. 2009, Crespo et al. 2011). This appears not to be the case in Miramichi Bay, which does allow an exchange of water between the bay and the offshore, principally through the two major inlets, Portage Channel and Portage Gully (Reinson 1977). The water exchange between the inner bay and the Gulf has been estimated at 15% per tidal cycle; as well, the influx of freshwater from the extensive Miramichi watershed results in a residual seaward drift of water, at least in the upper water column layers (Bousfield 1955).

Elsewhere, the study of *Alexandrium* cyst distribution and abundance in sediments has provided mixed results for forecasting the intensity of blooms the following growth season. Several successes have been described. Cembella et al. (1988) argued that *A. tamarense* cysts along the northern shore of the St. Lawrence Estuary initiated the toxic blooms that cause PSP on the south shore and further downstream in the estuary, although nothing was indicated about a relationship between cyst density and bloom intensity. Similarly, *A. tamarense* cyst accumulations in the Firth of Forth, on the northeast coast of Britain, were linked to toxic blooms in the adjacent coastal waters to the north (Lewis et al. 1995).

The greatest success at establishing such a link has been for the Gulf of Maine during 2005–2009, when the abundance of resting cysts in bottom sediments from the preceding autumn was a first-order predictor of the overall severity of *Alexandrium fundyense* blooms, as well as the extent of PSP closures along the coast, during the following of spring-summer (Anderson et al. 2005a, 2005b). For example, the ~50% drop in cyst abundance off the mid-coast of Maine in autumn 2005, led to a prediction that the 2006 bloom would be about half as bad as in 2005, which proved to be correct (Li et al. 2009).

The cyst abundance to bloom intensity relationship fell apart, however, for the summer of 2010, in coastal areas of the Gulf of Maine; in spite of a high cyst abundance in the autumn of 2009, the 2010 bloom intensity was low (McGillicuddy et al. 2011). In this case, environmental conditions in spring and summer 2010 were not favourable for growth of *A. fundyense*. Because of a large-scale change in circulation, near-surface waters were warmer, more stratified, and had both a lower salinity and a lower nutrient concentration, thus preventing cell proliferation.

In another study, new *A. tamarense* resting cysts were mapped following an unusually massive summer bloom in 2008, in the St. Lawrence Estuary (Gracia 2011). The August bloom, which originated at the mouth of the Saguenay River, closed fisheries and killed dozens of marine mammals (beluga whales and seals), fish and thousands of seabirds (Measures and Lair 2009, Dufour et al. 2010, Michael Scarratt, personal communication; see also: "Fatal Red Tide in

the St. Lawrence Estuary", at http://www.dfo-mpo.gc.ca/science/publications/annualreport-rapportannuel/ar-ra0809/sect2-eng.htm, as well as "2008 Red Tide Outcome and Conclusions", at http://www.qc.dfo-mpo.gc.ca/infoceans/201002/INFOCEANS-eng.pdf). Contrary to the original hypothesis (Garcia 2011), there was no strong spatial correlation between areas where shellfish toxicity was highest during the bloom and those where the highest cyst concentrations were observed 11 months after the event. Furthermore, the cyst concentrations detected at the beginning of July 2009 were not as high as expected, considering the abnormally massive *A. tamarense* bloom the previous year, and not even as high as cyst numbers were in 1988.

When there is a low correlation between one season's cyst abundance and bloom intensity the following season, both biological and physical factors must be taken into account to determine the success of the excystment in producing viable vegetative cells that may proliferate. First, not all cysts germinate in a season. For example, in Mill Pond, along the Massachusetts coastline, only 20% of the cysts in a 1-cm surface sediment layer germinated in a 6–8 week season (Crespo et al. 2011). Second, there can be a low viability of the cells just after excystment (see Fig. 6), as was the case in the Thau Lagoon, southern France; this confounded the relationship between cyst abundance and bloom intensity (Genovesi et al. 2009). Third, once the vegetative cells reach sunlit waters, they may stop growing if the temperature is not optimum or nutrients are limiting (McGillicuddy et al. 2011). Fourth, currents may divert the growing cells away from coast, thus avoiding contamination of shellfish stocks (McGillicuddy et al. 2011). Therefore, the final *Alexandrium* population size is not necessarily regulated by the abundance of cysts in bottom sediments, but rather by factors affecting cell growth and retention (Crespo et al. 2011). Nevertheless, the presence of cysts, as an inoculum, is necessary to start the bloom process at some initial location. In the case of Miramichi Bay, this location must be elsewhere.

By process of elimination, a remaining possibility is that the organisms are transported into the bay from offshore. Evidence for this is difficult to obtain, however. If toxic *A. tamarense* cells do originate offshore, they would be drawn into the bay by incoming tides, where they would toxify the shellfish, and then be removed rapidly from the bay by outgoing tides. Thus, sampling several days after such an event would miss the presence of toxic cells. We have experienced this on several occasions.

Alexandrium tamarense blooms occur frequently in the St. Lawrence Estuary (e.g. Blasco et al. 2003, Fauchot et al. 2008). An early study modelled the surface-layer transport of these cells from the St. Lawrence Estuary, "downstream" along the coast of the Gaspé Peninsula to coastal New Brunswick (Trites and Drinkwater 1991). The model was consistent with the timing of shellfish toxicity, which was first detected at Trois-Pistoles, Quebec, on the south side of the St. Lawrence Estuary in April, 1988 (reported in Trites and Drinkwater 1991). Toxic shellfish then appeared in May along the Gaspé coast, in the Baie des Chaleurs in early June, along the northeastern coast of New Brunswick and on the western shore of Prince Edward Island by mid-June, and on the north shore of Prince Edward Island by late June (Fig. 2). Interestingly, the pattern of molluscan toxicity reported by Worms et al. (1993) from sampling along the northeastern New Brunswick coast in 1988 is consistent with this model, although these data were apparently unavailable to Trites and Drinkwater when they wrote their paper.

In contrast to the above, however, Blasco et al. (2003) did not observe a downstream progression of the timing of the toxic shellfish outbreaks from the head of the Lower Estuary towards the tip of the Gaspé Peninsula, in spite of the strong Gaspé current. The onset of mussel toxicity was, in fact, in the opposite direction.

The findings of Blasco et al. (2003) about a lack of an "upstream" source of toxic *Alexandrium* cells are supported by the outcome of the high toxicity event in August 2008, described above, for the St. Lawrence Estuary (Gracia 2011). It was anticipated that some effect of this bloom, even residual, might be manifested in Miramichi Bay. However, no PSP toxins were found in mollusc samples at that time, or later that season. Interestingly however, PSP toxins were in fact detected in Miramichi Bay and western Northumberland Strait molluscs in 2008, but this occurred in May and June, a full month before the St. Lawrence Estuary bloom.

We conclude that hydrodynamics complicates the relationships among cysts in bottom sediments, vegetative cells in the water and toxic bivalves. Hence, cyst mapping is unlikely to be of great use in Miramichi Bay and similar coastal embayments. Clearly, the ability to forecast toxic *Alexandrium* blooms along the northeast coast of New Brunswick and elsewhere in the southern Gulf of St. Lawrence will require more study.

Most importantly and most easily accomplished, the data from ongoing regulatory sampling and analysis of bivalve molluscs by food inspection authorities should be carefully archived and made available for scientific studies of long-term trends. This would shed some light on this recurring phenomenon and also help to refine strategies for the number, frequency and location of shellfish samples collected for monitoring purposes.

6. Acknowledgements

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Appendix Table A1. Complete list of Prohibition Orders for the closures of molluscan shellfish harvesting in the eastern New Brunswick and Northumberland Strait coasts, southern Gulf of St. Lawrence, caused by levels of PSP toxins above the regulatory limit (80 µg STXeq/100 g). When available, links are given to the Prohibition Order (underlined). Information from DFO's Conservation and Protection Branch.

Order number	Area described	Open / Closure	Order date	Order Revoked
GTN-1990-23	Waters of Kent Co., to 300 m from shore, Escuminac Point to Richibouctou Cape, NB	Closure		
GTN-1990-24	Shippagan Harbour, NB	Closure	30-Nov-90	
GTN-1990-25	Miramichi Bay, 300 m from Oak Point to Swinging Point, NB	Closure		
GTN-1990-26	Waters and foreshore 300 m Portage Island, NB	Closure	30-Nov-90	
GTN-1990-27	Northumberland Strait waters to 300 m from shore, Pointe aux Renards to Pointe Grand-Digue, NB	Closure	30-Nov-90	
GTN-1990-28	Waters of Kent Co., 300 m from shore, Point Escuminac to Richibouctou Cape, NB	Open		GTN-1990-23
GTN-1991-06	Waters of Kent Co., 300 m from Point Escuminac to Richibouctou Cape, NB	Open	6-Nov-91	GTN-1990-23
GTN-1991-07	Waters of Kent Co., 300 m from Point Escuminac to Richibouctou Cape, NB	Closure	6-Nov-91	
GTN-1991-08	Miramichi Bay, 300 m from Oak Point to Swinging Point, NB	Open	6-Nov-91	GTN-1990-25
GTN-1991-09	Miramichi Bay, 300 m from Oak Point to Swinging Point, NB	Closure	6-Nov-91	
GTN-1996-01	Youghall Beach, Bathurst Harbour, Belloni Point, Caraquet, NB	Close	26-Jun-96	
GTN-1996-02	Area closed by GTN-1996-01	Open	23-Jul-96	GTN-1996-01
GTN-2000-01	Baie-Sainte-Anne, NB	Closure	29-Jun-00	
GTN-2000-02	Baie-Sainte-Anne, NB	Open	3-Aug-00	GTN-2000-01
GTN-2004-001	Miramichi Bay, NB	Closure	23-Jun-04	
GTN-2004-002	Miramichi Bay, NB	Open	22-Jul-04	GTN-2004-01
GTN-2005-001	Belledune Harbour to Limestone Point, NB	Closure	24-May-05	
GTN-2005-002	Belledune Harbour to Limestone Point, NB (error corrected)	Closure	25-May-05	GTN-2005-001
GTN-2006-001	Miramichi Bay and section of Gloucester Co., NB	Closure	9-Jun-06	

Order number	Area described	Open / Closure	Order date	Order Revoked
GTN-2006-002	Escuminac to Shippagan (Miramichi Bay), NB	Closure	14-Jun-06	GTN-2006-001
GTN-2006-003	Escuminac to Campbellton Bridge (all NE coast of NB)	Closure	16-Jun-06	GTN-2006-002
GTN-2006-005	Caraquet Bay, Lameque Bay, St-Simon Sud Bay, St-Simon Nord Bay, Pokesudée Bay, NB	Open	5-Jul-06	GTN-2006-003
GTN-2006-006	Miramichi Bay (portion), Neguac Bay, Tabusintac Bay, NB	Open	6-Jul-06	GTN-2006-005
GTN-2006-007	Baie-Sainte-Anne to Escuminac area; Brantville to Shippagan Gully; Maisonnette to Campbellton – reduced PSP closure	Closure	12-Jul-06	GTN-2006-006
GTN-2006-008	Brantville to Shippagan Gully remains closed (open Maisonnette to Campbellton and Escuminac to Pointe Carr), NB	Open	13-Jul-06	GTN-2006-007
GTN-2006-009	Brantville to Shippagan Gully, NB	Open	3-Aug-06	GTN-2006-008
GTN-2007-002	Escuminac Point to Cap-Lumière, NB	Closure	1-Jun-07	
GTN-2007-004	Escuminac Point to Pokemouche Beach, NB	Closure	6-Jun-07	
GTN-2007-005	Escuminac Point to Cap-Lumière, NB	Open	7-Jun-07	GTN-2007-002
GTN-2007-008	Chamberlain Point to Escuminac Point; Pokemouche Beach to Chamberlain Point remains closed, NB	Open	22-Jun-07	GTN-2007-004
GTN-2007-010	Pokemouche Beach to Chamberlain Point, NB	Open	6-Jul-07	GTN-2007-008
GTN-2008-001	Escuminac Point to Cap-Lumière, NB	Close	22-May-08	
GTN-2008-002	Pokemouche Beach to Chamberlain Point, NB	Close	23-May-08	
GTN-2008-003	Escuminac Point to Cap-Lumière, NB	Open	23-May-08	GTN-2007-005
GTN-2008-004	Escuminac Point to Chamberlain Point, NB	Close	30-May-08	
GTN-2008-005	Pokemouche Beach to Chamberlain Point, NB	Open	5-Jun-08	GTN-2008-002
GTN-2008-006	Escuminac Point to Cap-Lumière, NB	Open	11-Jun-08	GTN-2008-001
GTN-2008-007	Decreases size of closure: Escuminac Point to Portage Island to Rivière-des-Caches, NB	Close	11-Jun-08	GTN-2008-004
GTN-2008-008	Escuminac Point to Portage Island to Rivière-des-Caches, NB	Open	20-Jun-08	GTN-2008-007